SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
Unclassified	None

DTIC FILE COPY

AD-A212 627

3. DISTRIBUTION/AVAILABILITY OF REPORT
Approved for public release distribution unlimited.

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
Dept of Medical Neurosciences	SDRD-UWI-B	Walter Reed Army Institute of Research

6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
Walter Reed Army Institute of Research Washington, DC 20307-5100	Washington, DC 20307-5100

DTIC ELECTE SEP 21 1989 S E D

8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
USAMRDC		

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
	PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
Fort Detrick, Frederick, MD 21701	61102A	3M161102	S15CD	215

11. TITLE (Include Security Classification)
(U) Dexamethasone suppresses ACTH release without attenuating pituitary cyclic AMP response to stress in VIVO.

12. PERSONAL AUTHOR(S)
G.J. Kant, E.H. Mougey, A.J. Brown, and J.L. Meyerhoff

13a. TYPE OF REPORT	13b. TIME COVERED FROM _____ TO _____	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Dexamethasone, Stress, ACTH, Rat, Pituitary, Cyclic AMP, Glucocorticoid; biochemistry; (RT)a

19. ABSTRACT (Continue on reverse if necessary and identify by block number)
Dexamethasone, a synthetic glucocorticoid, has been shown to decrease basal and stress-elevated levels of the pituitary hormone ACTH. Glucocorticoids are known to bind to multiple sites within the brain and pituitary and it is not known which site(s) is most important in mediating the observed inhibition of ACTH release. At the level of the corticotroph, there is contradictory data from in vitro studies regarding whether dexamethasone acts proximal or distal to the formation of the cyclic AMP second messenger that has been shown to be involved in CRF-stimulated ACTH release. In the present report, we have examined the effects of dexamethasone pretreatment on stress-induced elevations in pituitary cyclic AMP and the release of ACTH in vivo.

Acute stress (15 min of intermittent footshock) elevated levels of pituitary cyclic AMP and plasma ACTH consistent with previous studies. Dexamethasone administration (0.4 mg/kg 24 hr prior to sacrifice plus 0.2 mg/kg 2 hr prior to sacrifice) inhibited stress-induced elevations in plasma ACTH but did not affect pituitary cyclic AMP response to acute stress. These findings suggest that dexamethasone inhibits the release of ACTH via an action distal to the generation of cyclic AMP. Keywords: reprints;

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
G. Jean Kant	(202) 576-3037	

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

Life Sciences, Vol. 45, pp. 125-131 Pergamon Press
Printed in the U.S.A.

DEXAMETHASONE SUPPRESSES ACTH RELEASE WITHOUT ATTENUATING PITUITARY CYCLIC AMP RESPONSE TO STRESS IN VIVO

G. Jean Kant, Edward H. Mougey, Angela J. Brown and James L. Meyerhoff

Department of Medical Neurosciences, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington DC 20307-5100[1,2]

(Received in finel form May 4, 1989)

Summary

Dexamethasone, a synthetic glucocorticoid, has been shown to decrease basal and stress-elevated levels of the pituitary hormone ACTH. Glucocorticoids are known to bind to multiple sites within the brain and pituitary and it is not known which site(s) is most important in mediating the observed inhibition of ACTH release. At the level of the corticotroph, there is contradictory data from in vitro studies regarding whether dexamethasone acts proximal or distal to the formation of the cyclic AMP second messenger that has been shown to be involved in CRF-stimulated ACTH release. In the present report, we have examined the effects of dexamethasone pretreatment on stress-induced elevations in pituitary cyclic AMP and the release of ACTH in vivo.

Acute stress (15 min of intermittent footshock) elevated levels of pituitary cyclic AMP and plasma ACTH consistent with previous studies. Dexamethasone administration (0.4mg/kg 24 hr prior to sacrifice plus 0.2mg/kg 2 hr prior to sacrifice) inhibited stress-induced elevations in plasma ACTH but did not affect pituitary cyclic AMP response to acute stress. These findings suggest that dexamethasone inhibits the release of ACTH via an action distal to the generation of cyclic AMP.

Since the purification and characterization of the hypothalamic peptide CRF in 1981 by Vale and colleagues, numerous in vitro and in vivo studies have shown that CRF is the primary physiological regulator of ACTH release (1,2). Furthermore, in vitro studies have shown that CRF-stimulated ACTH release from pituitary corticotrophs occurs via a series of steps initiated by binding of CRF to corticotroph receptors, followed by activation of adenylate cyclase, synthesis of cyclic AMP, activation of cyclic AMP dependent protein kinase, phosphorylation of corticotroph proteins and culminating in the release of ACTH (3,4).

---

1. The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3, AR 360-5).
2. Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NIH publication 85-23. All procedures were reviewed and approved by the WRAIR Animal Use Review Committee.

QUALITY INSPECTED 4

0024-3205/89 $3.00 +.00

By
Distribution/
Availability Codes
Dist | Avail and/or Special
A-1 20

89 9 19 094

Glucocorticoids, including the synthetic glucocorticoid dexamethasone, have been shown to inhibit the release of ACTH from pituitary cells in culture and to suppress resting and stress-induced ACTH release in vivo (5-12). There have been inconsistent reports from in vitro studies regarding the probable site(s) of action of dexamethasone in the pituitary. Some data suggest that dexamethasone acts proximal to the generation of cyclic AMP while other data suggest that dexamethasone acts distal to the formation of this second messenger (6,8). Some of these inconsistent findings may be due to the different in vitro test conditions employed.

Our laboratory has reported that acute stress increases levels of pituitary cyclic AMP in vivo and that this stress effect is highly correlated with the in vivo release of the three POMC-derived hormones regulated by CRF, i.e. ACTH, β-endorphin and β-LPH (13-18). In addition, stress-induced increases in levels of pituitary cyclic AMP and plasma ACTH are inhibited by administration of CRF antisera and blocked by lesions of the CRF-containing paraventricular nucleus of the hypothalamus (19,20). From these experiments and others, we have concluded that the observed stress-induced increase in pituitary cyclic AMP is the result of stress-released CRF activation of pituitary corticotroph CRF receptors and that cyclic AMP is involved in stress-induced release of ACTH and related POMC peptides (18-20).

Using the stress-induced elevations in pituitary cyclic AMP as an indicator, the present experiments were conducted to determine whether the pituitary site of action of dexamethasone was proximal or distal to cyclic AMP synthesis. Rats were pretreated with dexamethasone or vehicle and then sacrificed immediately upon removal from their home cage or following 15 min of intermittent footshock. The effects of dexamethasone pretreatment on pituitary cyclic AMP and plasma ACTH levels were then compared.

Materials and Methods

Animals

Male Sprague-Dawley rats (300 ± 50 grams) were purchased from Zivic-Miller and housed for a minimum of two weeks in our animal housing facility prior to each experiment. The rats were individually caged and maintained under a 12:12 lighting regimen with lights on at 0700 hrs. Food and water were freely available.

Drugs

Dexamethasone (Sigma, St. Louis, MO) was dissolved in a 5% ethanol/ 95% saline solution. Rats were injected twice with dexamethasone, once 24 hr prior to sacrifice with 0.4mg/kg and a second injection of 0.2mg/kg given 2 hrs prior to sacrifice. This dose regimen has been shown to suppress basal and stress-elevated ACTH levels (21). Vehicle-injected rats received the same amount of the ethanol/saline solution.

Footshock

Rats were either sacrificed immediately upon removal from their home cage or exposed to 15 min of intermittent footshock and sacrificed immediately following the 15 min stress session. Scrambled footshock was delivered on a variable interval schedule (VI 30) such that a 5 sec shock (1.6 mA intensity) was delivered approximately once per 30 sec. This stress model has been well-characterized in our laboratory (13-17).

Experimental Procedures

Two experiments were performed. In the first experiment, rats were sacrificed by decapitation to avoid any possibility of heat-induced degradation of ACTH that might result from microwave irradiation. In the second experiment, rats were sacrificed by microwave irradiation to prevent potential post-mortem changes in pituitary cyclic AMP levels. In each experiment, 12 rats were pretreated with dexamethasone and 12 rats with vehicle as described above. Six rats from each pretreatment group were sacrificed immediately upon removal from their home cage (controls) while another six rats from each pretreatment group were exposed to 15 min of footshock and then sacrificed. Following decapitation in the first experiment, whole pituitaries were dissected, weighed and quickly heated to 90° in sodium acetate buffer (pH 6.2, 0.05M) to prevent post-mortem changes in levels of cyclic AMP (22). Whole pituitaries were used to minimize dissection time; however, stress-induced increases in pituitary cyclic AMP are confined to the anterior lobe (23). After 15 min at 90°, the pituitaries were sonicated and centrifuged. Supernatants were stored until assayed for cyclic AMP. Trunk blood was collected with heparin and trasylol (a protease inhibitor). Following centrifugation, plasma was stored at -40° until assayed for ACTH.

In the second experiment, animals were sacrificed by microwave irradiation (5 sec of high power irradiation using a modified Varian PPS-2.5 power generator with an output of 2.5 KW at a frequency of 2450 MHz) to ensure that no post-mortem changes occurred in levels of pituitary cyclic AMP. In all other respects this experiment was identical to the first experiment. Pituitaries were placed in 1 ml sodium acetate buffer, sonicated and centrifuged as above. No 90° incubation was required since microwave irradiation inactivates enzymes in situ (22).

Radioimmunoassay Procedures

Cyclic AMP was determined by radioimmunoassay using antibodies produced in rabbits in our laboratory (24). A highly specific antiserum was used at a final dilution of 1:400,000. The antiserum exhibited cross-reactivities for ATP and GMP of less than 0.00007 and 0.14% respectively. Within assay variation was 7% and between assay variation was 18%.

ACTH was measured in unextracted rat plasma using a radioimmunoassay kit (Immuno Nuclear Corp). We have found that ACTH levels determined in extracted vs unextracted plasma from rat (unpublished data) but not human blood are equivalent (25). Human ACTH was used as a standard. The assay was performed in 12 x 75 polypropylene tubes using an overnight incubation at 4°C. Assay sensitivity was approximately 10 pg/ml. The intraassay coefficient of variation was 2.5% at 380 pg/ml and the interassay coefficient of variation was <5%. The cross-reactivity of the ACTH antibody (measured at 1200 pg/ml) is <0.01% with alpha-MSH, β-endorphin, β-LPH, Met or Leu-enkephalin.

Statistics

The data for each experiment were analyzed by two-way analysis of variance for the effects of pretreatment (vehicle or dexamethasone) and experimental group (control or footshock). Following the finding of a significant F score, selected follow-up comparisons between dexamethasone-treated control vs vehicle control and between dexamethasone-shocked and vehicle-shocked were made using Student's t test with Bonferroni correction.

Results

As shown in Fig 1., footshock increased levels of pituitary cyclic AMP in the first experiment ($F=27$, $df=1$, $p<0.0001$). Dexamethasone pretreatment did not alter pituitary cyclic AMP levels ($F=1.06$, $df=1$, $p>0.05$). As shown in Fig. 2 , footshock increased levels of plasma ACTH ($F=24$, $df=1$, $p<0.0001$) and dexamethasone pretreatment significantly decreased levels of ACTH ($F= 23$, $df=1$, $p<0.0001$).

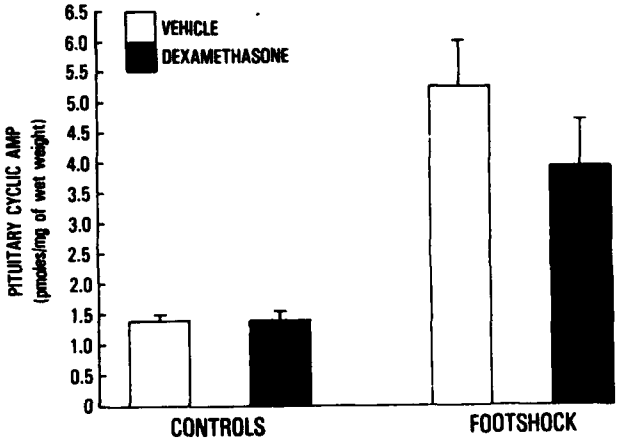

Fig 1.

Experiment 1. Effect of dexamethasone pretreatment on pituitary cyclic AMP response to acute stress (15 min intermittent footshock). Values represent the means ± SEM of 6 animals per group. Animals sacrificed by decapitation.

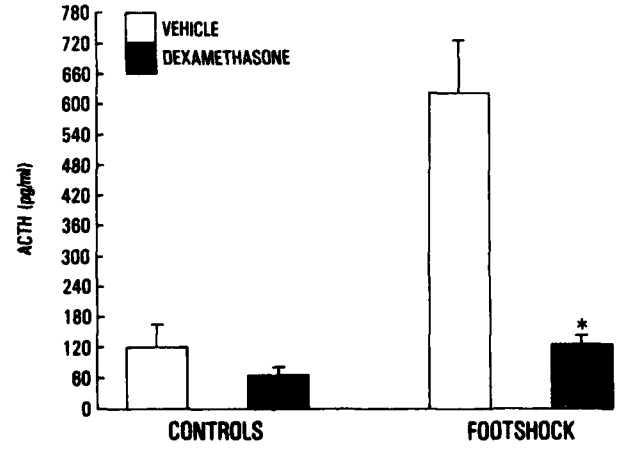

Fig 2.

Experiment 1. Effect of dexamethasone pretreatment on plasma ACTH response to acute stress. Values represent the means ± SEM of 6 animals per group. * significantly less than vehicle-pretreated from same experimental group, Student's t test with Bonferroni correction ($p<0.01$).

In experiment 2, rats were sacrificed by microwave irradiation. As in experiment 1, footshock increased levels of pituitary cyclic AMP ($F=13$, $df=1$, $p<0.01$), and dexamethasone treatment was without effect (Fig 3).

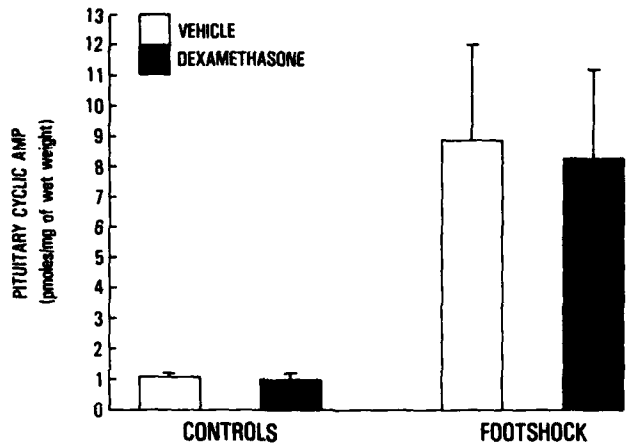

Fig 3.

Experiment 2. Effect of dexamethasone *pretreatment* on pituitary cyclic AMP response to acute stress. Values represent the means ± SEM of 6 animals per group. Animals sacrificed by microwave irradiation.

Discussion

The hypothalamic peptide CRF is thought to be the primary regulator for the control of pituitary ACTH release. The release of ACTH from pituitary corticotrophs in response to CRF stimulation has been shown to proceed via a series of steps in which cyclic AMP is involved as a second messenger. ACTH, in turn, stimulates the release of glucocorticoids (corticosterone, in rats) from the adrenal gland. Glucocorticoids have been shown to inhibit the release of ACTH. Both brain and pituitary sites of glucocorticoid binding have been described (26-29) and glucocorticoids have been shown to both inhibit CRF release from the hypothalamus and inhibit CRF binding to corticortrophs (29,30). Clearly, a brain site of action for the mechanism of glucocorticoid inhibition of ACTH release would be proximal to the CRF-stimulated generation of pituitary cyclic AMP. In the pituitary, glucocorticoids could inhibit ACTH release by actions either proximal (e.g at the CRF receptor) or distal (e.g. by affecting POMC expression) to cyclic AMP formation. The data generated by in vitro studies have not been consistent in this regard.

For example, the data of Bilezikjian and Vale suggest that dexamethasone inhibits both CRF-stimulated cyclic AMP production and ACTH release from cultured anterior pituitary cells. On the other hand, Giguere et al., also using a cultured anterior pituitary cell preparation, have reported that CRF stimulation of ACTH release was markedly inhibited by dexamethasone while CRF-stimulated cyclic AMP accumulation was unaffected.

Furthermore, while in vitro studies are useful in dissecting components of the physiological mechanisms involved in ACTH release, extrapolation of the results to the physiology of the intact animal with the hypothalamus, pituitary and adrenals working in concert is tenuous. Clearly, glucocorticoids bind to multiple sites. But which sites

are of most importance in inhibiting ACTH release in vivo ? The present study was conducted to attempt to answer this question.

Although stress causes the release of numerous central and peripheral neurotransmitters, releasing factors and hormones that might act upon different pituitary cell types (e.g. lactotrophs, somatotrophs) that utilize cyclic AMP as a second messenger, we have shown in a series of published experimental reports (18-20) that stress-induced elevations of pituitary cyclic AMP are the result of CRF stimulation of pituitary corticotroph receptors linked to adenylate cyclase. Stress-induced pituitary cyclic AMP elevations are highly correlated with increased plasma levels of corticotroph hormones regulated by CRF, i.e. ACTH, β-endorphin, β-LPH (17,18), but not with prolactin and growth hormone (15,18). Also, the pituitary cyclic AMP response to stress is mimicked by administration of exogenous CRF, is completely abolished by lesions of the major CRF cell body nucleus , the paraventricular nucleus of the hypothalamus (20) and is attenuated by pretreatment with CRF antisera (19). These data support numerous in vitro studies which have shown that CRF-stimulated release of corticotroph hormones utilizes cyclic AMP as a second messenger (3,4). Acute stress increases levels of pituitary cyclic AMP and plasma levels of corticotroph hormones in a parallel fashion. The present study was conducted to determine whether dexamethasone would inhibit both stress-induced pituitary cyclic AMP and plasma hormone elevations thus suggesting that the physiologically relevant in vivo site of action of dexamethasone was proximal to cyclic AMP formation, or whether dexamethasone would inhibit hormonal response to stress without affecting the cyclic AMP response thus suggesting that the primary site of action for dexamethasone was distal to cyclic AMP formation in the pituitary.

As in our previous studies, we found that 15 min of intermittent footshock produced robust increases in levels of pituitary cyclic AMP and plasma ACTH. Also, as has been reported by others, we found that dexamethasone suppressed stress-induced release of ACTH. We now report for the first time that dexamethasone does not suppress stress-induced elevations in pituitary cyclic AMP levels in vivo.

These in vivo data are in accord with in vitro experiments that have shown that dexamethasone inhibits ACTH release stimulated by exogenous cyclic AMP which suggests that dexamethasone acts, at least in part, on sites distal to cyclic AMP formation. The present in vivo experiments as well as the in vitro experiments cited (6,7) examined the effects of relatively long-term (18-24 hr) exposure to dexamethasone on mechanisms of release of corticotroph hormones. This length of exposure appears to affect hormonal release at a site distal to cyclic AMP formation; perhaps, by acting to decrease ACTH synthesis. Glucocorticoids have been shown to regulate proopiomelanocortin gene expression in vivo (31). Other glucocorticoid binding sites may play a more major role in mediating the rapid feedback effects of endogenously released glucocorticoids in response to stress or other stimuli.

Acknowledgements

The authors wish to thank David Collins, Clint Wormley, Lee Pennington, Willie Gamble, Clyde Kenion, Golden Driver, Leigh Landman-Roberts, Terry Eggleston and Paul D'Angelo for expert technical assistance and Karen Daniel for excellent secretarial support.

References

1. C. RIVIER and W. VALE, Nature 305, 325-327 (1983).
2. W. VALE, J. VAUGHAN, M. SMITH, G. YAMAMOTO, J. RIVIER and C. RIVIER, Endocrinology 113, 1121-1131 (1983).
3. G. AGUILERA, J.P. HARWOOD, J.X. WILSON, J. MORELL, J.H. BROWN and K.J. CATT, J. biol. Chem. 258, 8039-8045 (1983).
4. F. LABRIE, R. VEILLEUX, G. LEFEVRE, D.H. Coy, J. SUEIRAS-DIAZ and A.V. SCHALLY, Science 216, 1007-1008 (1982).

5. A. ABOU-SAMRA, K.J. CATT and G. AGUILERA, Endocrinology 119, 972-977 (1986).
6. L. M. BILEZIKJIAN and W.W. VALE, Endocrinology 113, 657-662 (1983).
7. M. CARNES, C.M. BARKSDALE, N.H. KALIN, M.S. BROWNFIELD and S.J. LENT, Neuroendocrinology 45, 160-164 (1987).
8. V. GIGUERE, F. LABRIE, J. COTE, D.H. COY, J. SUEIRAS-DIAZ and A.V.SCHALLY, Proc. Natl. Acad. Sci. 79, 3466-3469 (1982).
9. J. KRAICER and J.V. MILLIGAN, Endocrinology 87, 371 (1970).
10. S. NICHOLSON, E. CAMPBELL, A. TORRELLAS, R. BECKFORD, R. SANDFORD, R. SCRAGGS, B. GILLHAM and M. JONES, Neuroendocrinology 39, 343-349 (1984).
11. T. SUDA, N. TOMORI, F. TOZAWA, T. MOURI, H. DEMURA and K. SHIZUME, Endocrinology 114, 851-854 (1984).
12. E.P. WIDMAIER and M.F. DALLMAN, Endocrinology 115, 2368-2374 (1984).
13. G.J. KANT, B.N. BUNNELL, E.H. MOUGEY, L.L. PENNINGTON and J.L. MEYERHOFF, Pharmacol. Biochem. Behav. 18, 967-971 (1983).
14. G.J. KANT, T. EGGLESTON, L. LANDMAN-ROBERTS, C.C. KENION, G.C. DRIVER and J.L. MEYERHOFF, Pharmacol. Biochem. Behav. 22, 631-634 (1985).
15. G.J. KANT, R.H. LENOX, B.N. BUNNELL, E.H. MOUGEY, L.L. PENNINGTON and J.L. MEYERHOFF Psychoneuroendocrinology 8, 421-428 (1983).
16. G.J. KANT, J.L. MEYERHOFF, B.N. BUNNELL and R.H. LENOX, Pharmacol. Biochem, Behav. 17, 1067-1072 (1982).
17. G.J. KANT, E.H. MOUGEY, L.L. PENNINGTON and J.L. MEYERHOFF, Life Sciences 33, 2657-2663 (1983).
18. G.J. KANT, E.H. MOUGEY and J.L. MEYERHOFF, Neuroendocrinology 43, 383-390 (1986).
19. G.J. KANT, M.A. OLESHANSKY, D.D. WALCZAK, E.H. MOUGEY and J.L. MEYERHOFF, Peptides 7, 1153-1158 (1986).
20. J.L. MEYERHOFF, E.H. MOUGEY and G.J. KANT, Neuroendocrinology 46, 222-230 (1987).
21. J. ROSSIER, E. FRENCH, C. RIVIER, T. SHIBASAKI, R. GUILLEMIN and F.E. BLOOM, Proc. Natl. Acad. Sci. USA 77, 666-669 (1980).
22. R.H. LENOX, G.J. KANT and J.L. MEYERHOFF, Handbook of Neurochemistry, Vol 2, (Lajtha A., Ed) Plenum, New York, 77-102 (1982).
23. E.H. MOUGEY, J.L. MEYERHOFF, L.L. PENNINGTON, W.W. GAMBLE, G.C. DRIVER and G.J. KANT, Neuroscience Abstracts 12, 1059 (1986).
24. R.H. LENOX, G.J. KANT, G.R. SESSIONS, L.L. PENNINGTON, E.H. MOUGEY and J.L. MEYERHOFF, Neuroendocrinology 30, 300-308 (1980).
25. E.H. MOUGEY, L.M. LAMBE, J.L. MEYERHOFF, and G.J. KANT, Neuroscience Abstracts 14, 445 (1988).
26. B. BOHUS, E.R. DE KLOET and H.D. VELDHUIS, Current topics in neuroendocrinology, Vol 2, Adrenal actions on brain (Ganten D. and Pfaff D., Eds) Springer-Verlag, Berlin, 107-148 (1982).
27. M.T. JONES, B. GILLHAM, B.D. GREENSTEIN, U. BECKFORD and M.C. HOLMES, Current topics in neuroendocrinology, Vol 2, Adrenal actions on brain (Ganten D. and Pfaff D., Eds) Springer-Verlag, Berlin, 45-68 (1982).
28. B.S. MCEWEN, Current topics in neuroendocrinology, Vol 2, Adrenal actions on brain. (Ganten D. and Pfaff D., Eds) Springer-Verlag, Berlin, 1-22 (1982).
29. J. SCHWARTZ, N. BILLESTRUP, M. PERRIN, J. RIVIER and W. VALE, (1986) Endocrinology 119, 2376-2382 (1986).
30. S. NICHOLSON, E. CAMPBELL, A. TORELLAS, U. BECKFORD, R. ALTAHER, R. SANDFORD, R. SCRAGGS, B. GILLHAM and M. JONES, Neuroendocrinology 39 343-349 (1984).
31. N.C. BIRNBERG, J.C. LISSITZKY, M. HINMAN and E. HERBERT, Proc. Natl. Acad. Sci. USA 80, 6982-6986 (1983).